

Phylogenetic Relationships Among Species of Southwest Asian Leaf-toed Geckos (*Asaccus*)

Theodore J. Papenfuss^{1,6}, Todd R. Jackman², Aaron M. Bauer^{2,6}, Bryan L. Stuart³,
Michael D. Robinson⁴, and James F. Parham^{5,6}

¹ Museum of Vertebrate Zoology, University of California, Berkeley, CA 94720, USA; Email: asiaherp@berkeley.edu; ² Department of Biology, Villanova University, Villanova, PA 19085, USA; ³ North Carolina Museum of Natural Sciences, 11 W. Jones St., Raleigh, NC 27601, USA; ⁴ Department of Biology, Sultan Qaboos University, P.O. Box 36, Al Khoud, PC 123, Muscat, Oman; ⁵ Alabama Museum of Natural History, University of Alabama, Box 870340, Tuscaloosa, AL 35487, USA; ⁶ Research Associate, Department of Herpetology, California Academy of Sciences, 55 Music Concourse Drive, San Francisco, CA 94118, USA.

The first molecular phylogenetic analysis of *Asaccus* reveals high sequence divergence among the sampled species. *Asaccus montanus* is recovered as the sister to all other species. Two Arabian species (*A. gallagheri*, *A. platyrhynchus*) form a monophyletic group as suggested earlier by morphological analyses. Our data show that *A. gallagheri* from Nizwa, Oman likely represents a new species. Samples of two species (*A. elisae*, *A. griseonotus*) from north of the Persian Gulf form a well-supported clade. The traditional concept of *A. elisae* likely represents more than one species, as the samples included here are paraphyletic with respect to *A. griseonotus*. The taxonomy of *A. elisae* awaits comparison with samples from the type locality in Iraq. Our inability to resolve the phylogenetic position of two species (*A. caudivolvulus* and *A. nasrullahi*) prevents testing biogeographic hypotheses involving the occurrence of *Asaccus* on either side of the Persian Gulf.

The gecko genus *Asaccus* Dixon and Anderson 1973 (Squamata: Gekkonidae) contains 10 currently recognized species of leaf-toed geckoes (Fig. 1). *Asaccus* are distributed in northeastern Arabia (Oman, U.A.E.) and the Zagros Mountains (Iran, Iraq) (Fig. 2). One widespread species extends into Turkey and Syria. Although species have been described from 1895 to 2009 (see Taxonomic History below, Fig. 3), the only previous phylogeny was based on morphological characters



FIGURE 1. Left: *Asaccus montanus* from Nizwa, Oman (photo by TJP). Right: *Asaccus gallagheri* from Khasab, Oman. (photo by JFP).

(Arnold and Gardner 1994). Here we provide the first molecular phylogenetic analysis of the genus *Asaccus* using mitochondrial and nuclear DNA. This phylogeny provides insights into cryptic species diversity among Southwest Asian leaf-toed geckos and, by comparing our study with those on other taxa, contributes to the emerging biogeographic patterns for reptiles in the Persian Gulf region.

TAXONOMIC HISTORY

The first described Southwest Asian leaf-toed gecko was *Phyllodactylus elisae* Werner, 1895 from the ruins of the ancient city of Nineveh (near Mosul, Iraq). At that time, all leaf-toed geckos were placed in the widespread genus *Phyllodactylus*, a waste-basket taxon that included representatives from Asia, Africa, and the New World (see Bauer et al. 1997). Over a decade later, a second species was described from Dezful, Khuzestan Province, Iran (*Phyllodactylus eugeniae* Nikolskii, 1907). However, Werner (1917) synonymized *P. eugeniae* with *P. elisae*, which was accepted by subsequent authors (e.g., Wettstein, 1951; Wermuth, 1965). For several decades thereafter, the taxonomy of Southwest Asian leaf-toed geckos remained stagnant, with only one species, *P. elisae*, being recognized (Fig. 3).

The early 1970s brought a new cycle of research and discovery to Southwest Asian geckos in general, and leaf-toed geckos in particular. First, leaf-toed geckos were discovered on the Arabian Peninsula. *Phyllodactylus gallagheri* Arnold, 1972 was described from Masafi, Trucial States (now United Arab Emirates). The next year, Dixon and Anderson (1973) separated Southwest Asian leaf-toed geckos into a new genus, *Asaccus* (type species: *P. elisae*). About *P. gallagheri*, they stated, "Although we have not examined specimens of this new taxa [*sic*], we suspect that it is yet another representative of the genus *Asaccus* and would therefore extend the range of the genus southward onto the Arabian Peninsula" (Dixon and Anderson, 1973:157). In that paper they also described a third species of *Asaccus*, *A. griseonotus*, from Iraq and Iran. Around the same time,

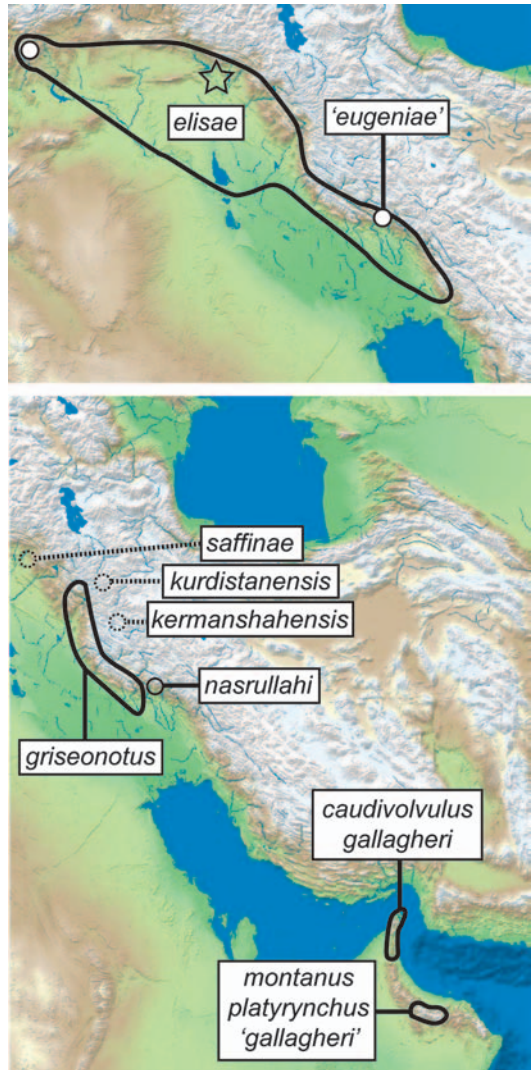


FIGURE 2. Map of samples used in this study. Top: The range of *Asaccus elisae*. The star indicates the type locality for the species in Iraq. The easternmost sample (circle) is from near the type locality of *eugeniae*, a species that may prove to be valid pending further study of the *A. elisae* complex. Bottom: Nine of the ten currently recognized species. *Asaccus 'gallagheri'* from Oman is placed in quotes because of the high sequence divergence from topotypic specimens from the Musandam Peninsula. Circles indicate samples used in this study. Three Zagros endemics (*kermanshahensis*, *kurdistanensis*, *saffinae*) are shown with dashed lines indicating their absence from this study.

Eiselt (1973) described *Asaccus ingae* from Lorestan Province, Iran. This species has been regarded as a junior synonym of *A. griseonotus* (Anderson, 1999:136) based on the month of description (December 1973 versus November 1973).

In 1977, Arnold reported the first records of *A. elisae* from the Arabian Peninsula from localities in the eastern United Arab Emirates and Oman, including the Musandam Peninsula of northern Oman and the Al Hajar Mountains nearly 300 kilometers southeast of the Musandam. Arnold (1977) noted extensive morphological variation among the samples of Arabian *A. elisae*, but postponed describing new species until specimens from the intervening 300 km could be examined. He later reiterated the distinctiveness of the Arabian samples, "Arabian populations of *Asaccus elisae* sen. lat. occur in two separate areas, each of which has distinct morphological characters. Both differ markedly from animals in Iraq and Iran and deserve at least subspecies status" (Arnold 1986:418). Arnold and Gardner (1994) later described these populations as *A. platyrhynchus* (Jebel Akhdar, Oman) and *A. caudivolvulus* (Musandam Peninsula region of U.A.E. and Oman). In the same year, Gardner (1994) reported a completely new species, *A. montanus*, from the Saiq Plateau of the Jebel Akhdar. Shortly thereafter, Rastegar-Pouyani (1996) described a new species from Iran *Asaccus kermanshahensis*, bringing the recognized species of *Asaccus* from three to seven between 1994 and 1996.

In just the past few years, three additional species have been described from Iran and Iraq: 1) *Asaccus nasrullahi* Werner, 2006 from the central Zagros Mountains of Lorestan Province, Iran (based on the reexamination of a specimen that was originally identified as *Ptyodactylus haselquistii* by Schmidt [1955]); 2) *Asaccus kurdistanensis* Rastegar-Pouyani, Nilson, and Faizi, 2006 from the Zagros Mountains in the Sarvabad region of Kurdistan Province, Iran; and 3) *Asaccus saffinae* Afrasiab and Mohamad, 2009 from a cave in the Irbil region of Iraqi Kurdistan.

To summarize, the diversity of the Southwest Asian leaf-toed geckos (now all referred to genus *Asaccus*) has been unfolding over the past century, although for most of this time, 1895-1972, only a single species was considered valid (Fig. 3). Given that molecular phylogenetics can often reveal hidden evolutionary lineages (e.g., Stuart et al. 2006; Parham and Papenfuss 2009), we expect that such studies, combined with traditional morphological analyses and fieldwork, will result in the recognition of even greater species diversity within this clade.

MATERIALS AND METHODS

Our study includes DNA sequence data from 12 museum vouchers at the California Academy of Sciences, San Francisco, California, USA (CAS) and the Museum of Vertebrate Zoology, University of California, Berkeley, California, USA (MVZ). Detailed locality information is available from these institutions. Species names, general localities, museum tissue and voucher numbers and GenBank numbers are presented in Table 1. Our samples represent seven of the 10 currently recognized species of *Asaccus*, lacking three species from Iran and Iraq (*A. kermanshahensis*, *A. kurdistanensis*, *A. saffinae*). Species names for the populations sampled were selected using the following criteria: (1) specimens that match the original morphological descriptions and were collected from localities given for holotype or paratypes (*A. caudivolvulus*, *A. griseonotus*, *A. montanus*,

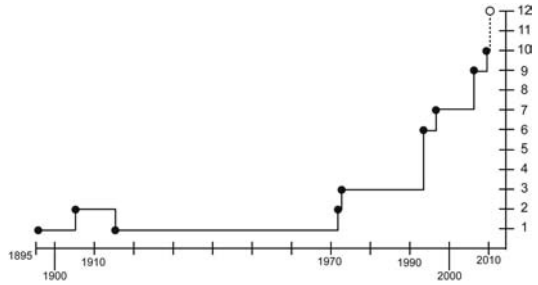


FIGURE 3. Number of species of *Asaccus* recognized through time. Dashed line and hollow circle show the postulated number of species based on the phylogeny and sequence divergence found in this study.

TABLE 1. Samples of *Asaccus* and *Haemodracon* sequenced in this study.

| Species | General Locality | Voucher number | Tissue Number | Genbank RAG-1 | Genbank ND2 |
|-----------------------------|-------------------|----------------|---------------|---------------|-------------|
| <i>H. riebeckii</i> | Socotra Island | MVZ 236486 | MVZ 236486 | HM212506 | HM212518 |
| <i>A. caudivolvulus</i> | Khasab, Oman | CAS 225372 | MVZ 241367 | HM212507 | HM212519 |
| <i>A. elisae</i> | Sanliurfa, Turkey | CAS 218142 | CAS 218142 | HM212509 | HM212521 |
| <i>A. elisae</i> | Sanliurfa, Turkey | CAS 218143 | CAS 218143 | HM212510 | HM212522 |
| <i>A. elisae "eugeniae"</i> | Khuzestan, Iran | MVZ 234315 | MVZ 234315 | HM212511 | HM212523 |
| <i>A. gallagheri</i> | Khasab, Oman | CAS 225358 | MVZ 241368 | HM212513 | HM212525 |
| <i>A. gallagheri</i> | Nizwa, Oman | CAS 225379 | MVZ 241371 | HM212516 | HM212528 |
| <i>A. griseonotus</i> | Lorestan, Iran | MVZ 234325 | MVZ 234325 | HM212512 | HM212524 |
| <i>A. griseonotus</i> | Lorestan, Iran | MVZ 234326 | MVZ 234326 | HM212508 | HM212520 |
| <i>A. montanus</i> | Nizwa, Oman | CAS 225399 | MVZ 241370 | HM212515 | HM212527 |
| <i>A. nasrullahi</i> | Khuzestan, Iran | MVZ 234330 | MVZ 234330 | HM212514 | HM212526 |
| <i>A. platyrhynchus</i> | Nizwa, Oman | CAS 225386 | MVZ 241372 | HM212517 | HM212529 |

A. platyrhynchus, *A. nasrullahi*) or (2) specimens identified using keys in Arnold and Gardner (1994) and collected from localities within the known range of a given species (*A. gallagheri*; *A. elisae*). The outgroup, *Haemodracon riebeckii* (Peters, 1882), from Socotra Island, is strongly supported to be sister to *Asaccus* within the Phyllodactylidae (Gamble et al. 2008).

Total genomic DNA was extracted from liver or muscle tissue using PureGene Animal Tissue DNA Isolation Protocol (Gentra Systems, Inc.). A fragment of mitochondrial DNA that encodes the complete NADH dehydrogenase subunit 2 gene and flanking tRNAs (simply 'ND2' hereafter) was amplified by the polymerase chain reaction (PCR; 94°C 45s, 48°C 30s, 72°C 1 min) for 35 cycles using the primer L4437 (Macey et al., 1997) and H5934 (Macey et al., 1997) or H-Alaliz (5'-AAAGTGTGTTGAGTTGCATTCA-3'). A fragment of nuclear DNA that encodes part of the recombination activating protein 1 (RAG-1) gene was amplified by PCR (94°C 45s, 55°C 30s, 72°C 1 min) for 35 cycles using the primers L-RAG1b and H-RAG1b (Kearney and Stuart, 2004). PCR products were electrophoresed in a 1% low melt agarose TALE gel stained with ethidium bromide and visualized under ultraviolet light. The bands containing DNA were excised and agarose was digested from bands using GELase (Epicentre Technologies). PCR products were sequenced in both directions by direct double strand cycle sequencing using the amplifying primers and Big Dye version 3 chemistry (Perkin Elmer). The internal primers L-snRAG1 and H-snRAG1 (Kearney and Stuart, 2004) were also used in the RAG-1 sequencing reactions. Cycle sequencing products were precipitated with ethanol, 3 M sodium acetate, and 125 mM EDTA, and sequenced with a Prism 3100 Genetic Analyzer (ABI) or 3730 DNA Analyzer (ABI). Sequences were edited using Sequencher v. 4.1 (Genecodes). Sequences were aligned using Geneious Pro 4.6 software (Biomatters, Auckland, New Zealand) and adjusted by eye. Phylogenetic relationships among the samples were assessed using Maximum Likelihood. The Akaike Information Criterion (AIC) in ModelTest 3.06 (Posada and Crandall, 1998) was used to find the model of evolution that best fit the data. The GTR + Γ + I model was used in GARLI 0.951 (Zwickl 2006) with simultaneous tree and parameter estimations. We used nonparametric bootstraps (1000 pseudoreplicates) to assess node support in resulting topologies.

RESULTS

A 1287 bp fragment of mtDNA (ND2) and a 1381 bp fragment of nuclear DNA (RAG-1) were obtained from all samples. The mitochondrial sequence divergence among species of *Asaccus* (Table 2) is extremely high (over 39% HKY corrected sequence divergence for ND2) and nearing saturation (see Discussion). The resultant phylogenetic trees are shown in Figs. 4B–D. Although RAG-1 is far less variable than ND2 (Table 2), the combined tree topology is the same as the RAG-1 topology (Fig. 4D), suggesting that the small number of informative sites contains far less noise than ND2. Increasing the length of RAG-1 sequences should improve support for branches in the combined tree. The only difference between the ND2 and RAG-1 tree is the placement of *A. nasrullahi*, which receives weak support as the sister to *A. caudivolvulus* in the ND2 tree (Fig. 4B) and is sister to all *Asaccus* except *A. montanus* in the RAG1 tree (Fig. 4C). Aside from the relationships of *A. nasrullahi*, all taxon clusterings within *Asaccus* receive high bootstrap values in the combined tree. In contrast to the previous phylogenetic hypothesis of *Asaccus* relationships (Arnold and Gardner 1994; Fig. 4A), our study recovered *A. montanus* as the sister to all other sampled species (Fig. 4B–D). Two Arabian species (*A. gallagheri* and *A. platyrhynchus*) form a monophyletic group as hypothesized by Arnold and Gardner (1994: Fig. 4A) on morphological characters (a caudal tubercle and sexually dimorphic tail color).

DISCUSSION

The high sequence divergence between species of *Asaccus* (Tables 2, 3) suggests that it is a very old clade. The HKY corrected sequence divergence between *Asaccus* and its sister taxon *Haemodracon* Bauer, Good, and Branch, 1997 is 31.3–37.6% (Table 2). Such high sequence divergence is nearly comparable to that among species of the sphaerodactylid gekko genus *Pristurus* Rüppell, 1835 (up to 42.4% in ‘ND2’, Papenfuss et al. 2009) which shares a distribution with the *Haemodracon* + *Asaccus* clade, occurring in both Oman and the island of Socotra. These high divergences certainly reflect saturation of the ND2 gene. Uncorrected distances between squamates and birds are 41.5% for the ND2 gene, indicating that 40% represents an approximate upper ceiling on meaningful distances for ND2.

Zoogeographically, *Asaccus* is one of several taxa that have closely related populations or species distributed on either side of the Persian Gulf (Arnold 1972). Rastegar-Pouyani et al. (2006) hypothesized that the Zagros was the center of origin of *Asaccus*, but our data show that *A. montanus* from Arabia is the most basal species. Unfortunately, further discussion of the biogeography of *Asaccus* across the Persian Gulf must await analysis of the three Zagros endemics (*A. kermanshahensis*, *A. kurdistanensis*, *A. saffinae*), and resolving the uncertain phylogenetic position of *Asaccus caudivolvulus* and *A. nasrullahi*.

In a few cases, our data indicate that current taxonomy is underestimating total diversity of *Asaccus*. The ND2 sequence divergence is very high (14.0%, Table 2) between the two samples of *A. gallagheri* from Nizwa and Khasab (Oman) implying that more than one species is present. Since our sample from Khasab is near to the type locality in the U.A.E., the divergent sample from Nizwa may represent a new species. Additional morphological study and fieldwork for geographically intervening samples are needed to delineate species boundaries for the putative taxa within *A. gallagheri*. Mitochondrial data also show that our Iranian sample of *A. elisae* is more closely related to *A. griseonotus* than it is to the Turkish samples of *A. elisae*. Clearly the concept of *A. elisae* represents more than one species. The Iranian sample is from near the type locality of *Phyllodactylus eugeniae*, which is currently in the synonymy of *A. elisae* (see Taxonomic History sec-

TABLE 2. HKY corrected distances for ND2 above the diagonal and uncorrected distances below.

| | | | | | | | | | | | | |
|--|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 <i>Asaccus caudivolvulus</i> MVZ241367 | — | 0.32663 | 0.32663 | 0.35698 | 0.34578 | 0.39492 | 0.33187 | 0.33471 | 0.39084 | 0.32313 | 0.41360 | 0.44215 |
| 2 <i>Asaccus elisae</i> CAS218142 | 0.25789 | — | 0.00000 | 0.18116 | 0.34621 | 0.37899 | 0.17312 | 0.17215 | 0.34132 | 0.31061 | 0.39655 | 0.42052 |
| 3 <i>Asaccus elisae</i> CAS218143 | 0.25789 | 0.00000 | — | 0.18116 | 0.34621 | 0.37899 | 0.17312 | 0.17215 | 0.34132 | 0.31061 | 0.39655 | 0.42052 |
| 4 <i>Asaccus elisae</i> "engeniae" MVZ234315 | 0.27601 | 0.15678 | 0.15678 | — | 0.27161 | 0.29328 | 0.15012 | 0.15629 | 0.39083 | 0.33245 | 0.43670 | 0.44991 |
| 5 <i>Asaccus gallagheri</i> MVZ241368 | 0.27186 | 0.27207 | 0.27207 | 0.27981 | — | 0.16018 | 0.35908 | 0.36320 | 0.42212 | 0.36503 | 0.35637 | 0.49132 |
| 6 <i>Asaccus gallagheri</i> MVZ241371 | 0.29881 | 0.28973 | 0.28973 | 0.29328 | 0.13977 | — | 0.39690 | 0.39822 | 0.46805 | 0.39674 | 0.37367 | 0.52984 |
| 7 <i>Asaccus griseonotus</i> MVZ234325 | 0.26178 | 0.15085 | 0.15085 | 0.13186 | 0.35908 | 0.39690 | — | 0.00637 | 0.37779 | 0.31367 | 0.40134 | 0.41463 |
| 8 <i>Asaccus griseonotus</i> MVZ234326 | 0.26366 | 0.15014 | 0.15014 | 0.13646 | 0.28213 | 0.30004 | 0.00633 | — | 0.38402 | 0.32270 | 0.40485 | 0.42434 |
| 9 <i>Asaccus montanus</i> MVZ241370 | 0.29922 | 0.26984 | 0.26984 | 0.29983 | 0.31601 | 0.33991 | 0.29109 | 0.29471 | — | 0.39185 | 0.49501 | 0.43831 |
| 10 <i>Asaccus nasrullahi</i> MVZ234330 | 0.25725 | 0.25085 | 0.25085 | 0.26434 | 0.28361 | 0.30141 | 0.25236 | 0.25780 | 0.29812 | — | 0.43297 | 0.42806 |
| 11 <i>Asaccus platyrhynchus</i> MVZ241372 | 0.30945 | 0.30027 | 0.30027 | 0.32287 | 0.27801 | 0.28717 | 0.30301 | 0.30542 | 0.35251 | 0.32251 | — | 0.53895 |
| 12 <i>Haemodracon riebeckii</i> MVZ236486 | 0.32834 | 0.31581 | 0.31581 | 0.33183 | 0.35453 | 0.37116 | 0.31318 | 0.31856 | 0.32444 | 0.32027 | 0.37607 | — |

TABLE 3. HKY corrected distances for RAG-1 above the diagonal and uncorrected distances below.

| | | | | | | | | | | | | |
|--|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 <i>Asaccus caudivolvulus</i> MVZ241367 | — | 0.01469 | 0.01617 | 0.01319 | 0.01546 | 0.01469 | 0.01467 | 0.01501 | 0.02139 | 0.01916 | 0.01394 | 0.04597 |
| 2 <i>Asaccus elisae</i> CAS218142 | 0.01452 | — | 0.00073 | 0.00146 | 0.01845 | 0.01767 | 0.00291 | 0.00235 | 0.02515 | 0.01765 | 0.01617 | 0.05239 |
| 3 <i>Asaccus elisae</i> CAS218143 | 0.01595 | 0.00073 | — | 0.00291 | 0.01991 | 0.01914 | 0.00436 | 0.00391 | 0.02665 | 0.01913 | 0.01764 | 0.05481 |
| 4 <i>Asaccus elisae</i> "engeniae" MVZ234315 | 0.01305 | 0.00145 | 0.00290 | — | 0.01691 | 0.01614 | 0.00145 | 0.00077 | 0.02361 | 0.01614 | 0.01540 | 0.05231 |
| 5 <i>Asaccus gallagheri</i> MVZ241368 | 0.01523 | 0.01814 | 0.01955 | 0.01665 | — | 0.00363 | 0.01690 | 0.01898 | 0.02516 | 0.02142 | 0.00729 | 0.05322 |
| 6 <i>Asaccus gallagheri</i> MVZ241371 | 0.01451 | 0.01741 | 0.01883 | 0.01593 | 0.00362 | — | 0.01614 | 0.01813 | 0.02513 | 0.02064 | 0.00582 | 0.05092 |
| 7 <i>Asaccus griseonotus</i> MVZ234325 | 0.01450 | 0.00290 | 0.00434 | 0.00145 | 0.01665 | 0.01593 | — | 0.00155 | 0.02511 | 0.01762 | 0.01539 | 0.05113 |
| 8 <i>Asaccus griseonotus</i> MVZ234326 | 0.01483 | 0.00234 | 0.00390 | 0.00077 | 0.01865 | 0.01786 | 0.00155 | — | 0.02461 | 0.01740 | 0.01655 | 0.05233 |
| 9 <i>Asaccus montanus</i> MVZ241370 | 0.02103 | 0.02465 | 0.02608 | 0.02317 | 0.02462 | 0.02462 | 0.02462 | 0.02414 | — | 0.02062 | 0.02438 | 0.05449 |
| 10 <i>Asaccus nasrullahi</i> MVZ234330 | 0.01885 | 0.01740 | 0.01884 | 0.01593 | 0.02100 | 0.02028 | 0.01738 | 0.01715 | 0.02028 | — | 0.01989 | 0.05207 |
| 11 <i>Asaccus platyrhynchus</i> MVZ241372 | 0.01378 | 0.01596 | 0.01738 | 0.01521 | 0.00724 | 0.00579 | 0.01521 | 0.01632 | 0.02390 | 0.01955 | — | 0.05085 |
| 12 <i>Haemodracon riebeckii</i> MVZ236486 | 0.04442 | 0.05039 | 0.05260 | 0.05031 | 0.05108 | 0.04897 | 0.04922 | 0.05033 | 0.05233 | 0.05002 | 0.04894 | — |

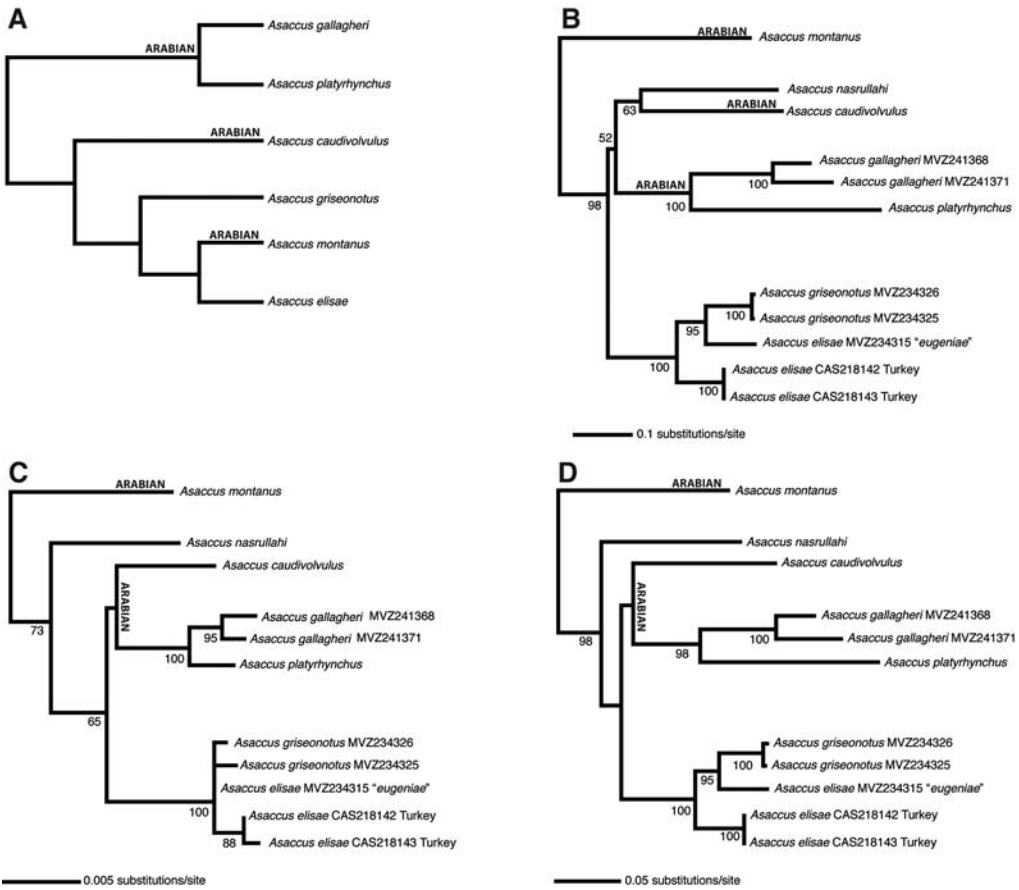


FIGURE 4. Phylogenetic hypotheses for the genus *Asaccus*. A: Phylogeny based on morphology (Arnold and Gardner, 1994); B-D: Maximum Likelihood analysis of mitochondrial DNA (ND2, B), nuclear DNA (RAG-1, C), and combined data (D).

tion above). Tok et al. (1997) and Baran et al. (2003) presented morphological data on *A. elisae* from Iraq, Iran, Syria, and Turkey and concluded that the observed variation was infraspecific. However, without examining or sequencing samples of topotypic *A. elisae* from Iraq it is difficult to know if the Iranian or Turkish samples studied here represent true *A. elisae* or should be referred to a new species. Likewise, although new populations of *Asaccus* continue to be discovered (Martens and Kock 1990; Franzen et al. 2002; Tok et al. 2002; Afrasiab and Mohamad 2009), our knowledge of the true distributional range of all members of the genus remains rudimentary and the geographic factors influencing species boundaries must remain speculative.

CONCLUSIONS

The first molecular phylogenetic analysis of *Asaccus* shows high sequence divergence among the sampled species. *Asaccus montanus* is revealed as the sister to all other species. Our inability to resolve the phylogenetic position of two species (*A. caudivolvulus* and *A. nasrullahi*) prevents the testing of biogeographic hypotheses involving the occurrence of *Asaccus* on either side of the Persian Gulf. Two Arabian species (*A. gallagheri*, *A. platyrhynchus*) form a monophyletic group as

suggested by morphological analyses. Our data show that *A. gallagheri* from Nizwa, Oman likely represents a new species. Samples of two species (*A. elisae*, *A. griseonotus*) from north of the Persian Gulf form a well-supported monophyletic clade. The traditional concept of *A. elisae* likely represents more than one species, as the samples included here are paraphyletic with respect to *A. griseonotus*. The taxonomy of *A. elisae* awaits comparison with samples from the type locality in Iraq. In addition to the species descriptions outlined above, future directions include adding the missing Zagros endemics (*A. kurdistanensis*, *A. saffinae*) as well as sequencing additional markers in order to resolve the existing polytomies.

ACKNOWLEDGMENTS

We thank Ali Al Kiyumi (Director of Conservation, Ministry of Environment and Climate Affairs) for granting Oman collecting and export permits. Collecting and export permits for Iran were issued by the Iran Department of Environment. Marycke Jongbloed assisted with fieldwork in Khasab, Oman. Houman Jowkar and members of Pars Herpetological Institute (Hadi Fahimi, Hanyeh Ghaffari, Kamran Kamili, Omid Mozaffari) assisted with fieldwork in Iran. We thank İsmail Uğurtaş (Uludağ University) for facilitating fieldwork in Turkey. Hadi Fahimi, Houman Jowkar, and Steve Anderson (University of the Pacific) helped with fieldwork in Turkey. Steve Anderson also provided valuable assistance with species identifications and the literature. W. Brian Simison (Center for Comparative Genomics at the California Academy of Sciences) assisted with lab work. Partial funding for fieldwork was provided by a grant from the George Lindsay Field Research Fund of the California Academy of Sciences. JFP was partially supported by the John D. and Catherine T. MacArthur Foundation funding of the Biodiversity Synthesis Group of the Encyclopedia of Life. Additional funding was provided by Grant DEB 0844523 to AMB and TRJ from the National Science Foundation.

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